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Cladorchis pyriformis (Diesing, 1838) (Digenea, Cladorchiidae): new rodent host and geographic records in Argentina

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Abstract

This work contributes to the knowledge of digeneans in two species of *Holochilus* Brant, 1835 from nine localities of Argentina. Morphological characteristics and ecological data are provided. *Holochilus vulpinus* (Brants, 1827) and *H. chacarius* Thomas, 1906 were parasitized by only one digenean species, *Cladorchis pyriformis* (Diesing, 1838). The morphometric features of this species and those reported by previous authors from South America are presented and compared. *Holochilus vulpinus* and a locality of Corrientes province was the host most frequently parasitized. *Cladorchis pyriformis* is recorded for the first time in *H. chacarius*.

Keywords

Cricetidae, digeneans, *Holochilus*, Rodentia.

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Introduction

Digeneans are common parasites of small mammals worldwide. Around 225 species of mammals are parasitized by trematodes in South America. Thirty families of trematodes have been reported from Rodentia (Feliú et al. 2006; Fernandes et al. 2015). Until now, seven species of digeneans were reported from Cricetidae in Argentina (Lunaschi and Drago 2007).

Nearby 50 surveys on digeneans from mammals have been published from wild mammals in Argentina. Only 28 mammals species (7%) grouped into 15 families have been reported to be parasitized by Digenea (Lunaschi and Drago 2007). Among the rodents, only one species of Muridae, *Rattus norvegicus* (Berkenhout, 1769) and seven of Cricetidae, *Akodon azarae* (Fischer, 1829), *Deltamys kempi* Thomas, 1917, *Holochilus vulpinus* (Brants, 1827), *Oxymycterus rufus* (Fischer, 1814), *Oligoryzomys flavescens* (Waterhouse, 1837), *Oligoryzomys nigripes* (Olfers, 1818), and *Scapteromys aquaticus* Thomas, 1920, have been reported as hosts of digeneans (Lunaschi and Drago 2007; Navone et al. 2009).

Among sigmodontinae rodents (Cricetidae), marsh rats of the genus *Holochilus* are large herbivorous and semiaquatic rodents. The genus is broadly distributed throughout riparian or marshy habitats in tropical lowlands of South America (Massoia 1971, 1976; Voglino et al. 2004; Gonçalves et al. 2015). *Holochilus* sp. has been recorded harboring 11 nematode species (e.g. Durette-Desset et al. 1997; Notarnicola 2005; Notarnicola et al.

2010; Digiani et al. 2013, 2015; Robles et al. 2018), and three digenean species, *Cladorchis pyriformis* (Diesing, 1838), *Urotrema scabridum* Braum, 1990, and *Schistosoma mansoni* Sambon, 1907 (Sutton and Lunaschi 1990; Picot 1992; Lunaschi and Drago 2007; Fernandes et al. 2015).

The present study contributes to the knowledge of the parasitism and distribution of digeneans in two *Holochilus* species from different localities of northwestern Argentina. Also, morphological characteristics and ecological data are provided.

Methods

The viscera of 29 marsh rats (10 *H. vulpinus* and 19 *H. chacarius* Thomas, 1906) were analyzed in order to find digenean specimens. Sigmodontinae rodents were trapped during various field studies by several collaborators (see Acknowledgements) between 1994 and 2018 in Argentina (Table 1, Fig. 1).

Digeneans found in the rodent's caecum were removed under a stereomicroscope (Olympus SZ61–TR), in some cases in the field and in other cases in the laboratory after the fixation of the viscera. Subsequently, digeneans were fixed in 5% formalin and preserved in 70% ethanol. Some specimens were stained with hydrochloric carmine, dehydrated in a graded ethanol series, cleared in eugenol, and mounted in natural Canada balsam. In addition, serial histological sections of three specimens were made for a complete study of the internal morphology. Whole specimens and sections were studied and photographed using a polarized light microscope (Olympus BX51®). Two specimens were dried using the critical point method, examined and photographed by scanning electron microscopy (SEM) (JEOL, JSM 6360 LV).

The ecological parameters prevalence (P), mean intensity (MI), and mean abundance (MA) were calculated according to Bush et al. (1997).

Measurements of species are presented in Table 2 as follows: mean, standard deviations, and range in parentheses. All measurements are given in millimeters (mm) unless otherwise indicated.

Helminths voucher specimens were deposited in the Helminthological Collection from Museo de La Plata (MLP-He 7522, 7523, 7524), La Plata, Buenos Aires and hosts in Mastozoological Collection from Museo de La Plata (MLP), MLP 27.XII.01.5, 27.XII.01.6, 27.XII.01.7 (*H. vulpinus* from Estancia San Juan de Poriahú-ESJP) and in Mastozoological Collection from Centro Nacional Patagónico (CNP), Puerto Madryn, Chubut, CNP 3720 (*H. chacarius* from Estación de Animales Silvestres Guaycolec–EASG). The catalog numbers of the collection are waiting to be assigned, field number CG 848, CG 866 (*H. vulpinus* from Villa Elisa-VE).

Results

New parasite records (Fig. 1). Argentina: Formosa province: Estación de Animales Silvestres Guaycolec (25°58′54″S, 58°09′58″W), Natalia Guerreiro Martins coll., (56 specimens). Argentina: Corrientes province: Estancia San Juan de Poriahú (27°42′06″S, 57°12′14″W), Maria del Rosario Robles coll., (30 specimens). Argentina: Entre Ríos province: Villa Elisa (32°08′00″S, 58°24′00″W), Natalia Guerreiro Martins coll., (19 specimens).

Both host species were parasitized by only one digenean species, which was identified as *Cladorchis pyriformis*. A total of 49 digenean specimens were found in five *H. vulpinus* analyzed (P = 50%, MI = 9.8, MA = 4.9), whereas 56 digenean specimens were found in only one *H. chacarius* analyzed (P =5.3%, MI = 56, MA = 2.9). The values of P, MI, and MA in the different sampling areas were: 100%, 10 and 10 for ESJP; 66.7%, 9.5 and 6.3 for VE; and 33.3%, 56 and 18.6 for EASG, respectively (Table 1).

Identification. The measurements of the present specimens and those reported by previous authors from South America are presented in Table 2.

The morphological characteristics of the specimens agree with those provided in the descriptions of *C. pyriformis* by Diesing (1838) and Fischoeder (1901), and with those observed by Sutton and Lunaschi (1990)

Table 1. List of sampled localities of *Holochilus chacarius* and *Holochilus vulpinus*.

Code	Localities	Latitude (S)	Longitude (W)	Host species (*n*)	
				H. chacarius	*H. vulpinus*
1	Instituto de Investigación para la Pequeña Agricultura Familiar del Noreste Argentino (IPAF NEA), Laguna Blanca, Pilcomayo Departament, Formosa province	25°12′00″	058°07′00″	13	–
2	Estacion de Animales Silvestres Guaycolec, Formosa Departament, Formosa province	25°58′54″	058°09′58″	3	–
3	Selvas de Rio de Oro, Libertador General San Martín Departament, Chaco province	26°46′51″	058°57′55″	3	–
4	Estancia San Juan Poriahú, San Miguel Departament, Corrientes province	27°42′06″	057°12′14″	–	3
5	Estancia San Nicolás, San Miguel Departament, Corrientes province	28°07′07″	057°25′34″	–	1
6	Estancia El Cimarrón, RP 118, km 96, Corrientes province	28°06′41″	057°52′21″	–	1
7	Paraje Atalaya, Santo Tomé Departament, Corrientes province	28°30′05″	056°02′04″	–	1
8	Villa Elisa, Colón Departament, Entre Ríos province	32°08′00″	058°24′00″	–	3
9	Punta Indio, Partido de Punta Piedras, Buenos Aires province	35°16′00″	057°15′00″	–	1

The code numbers correspond to the localities as indicated Fig. 1.

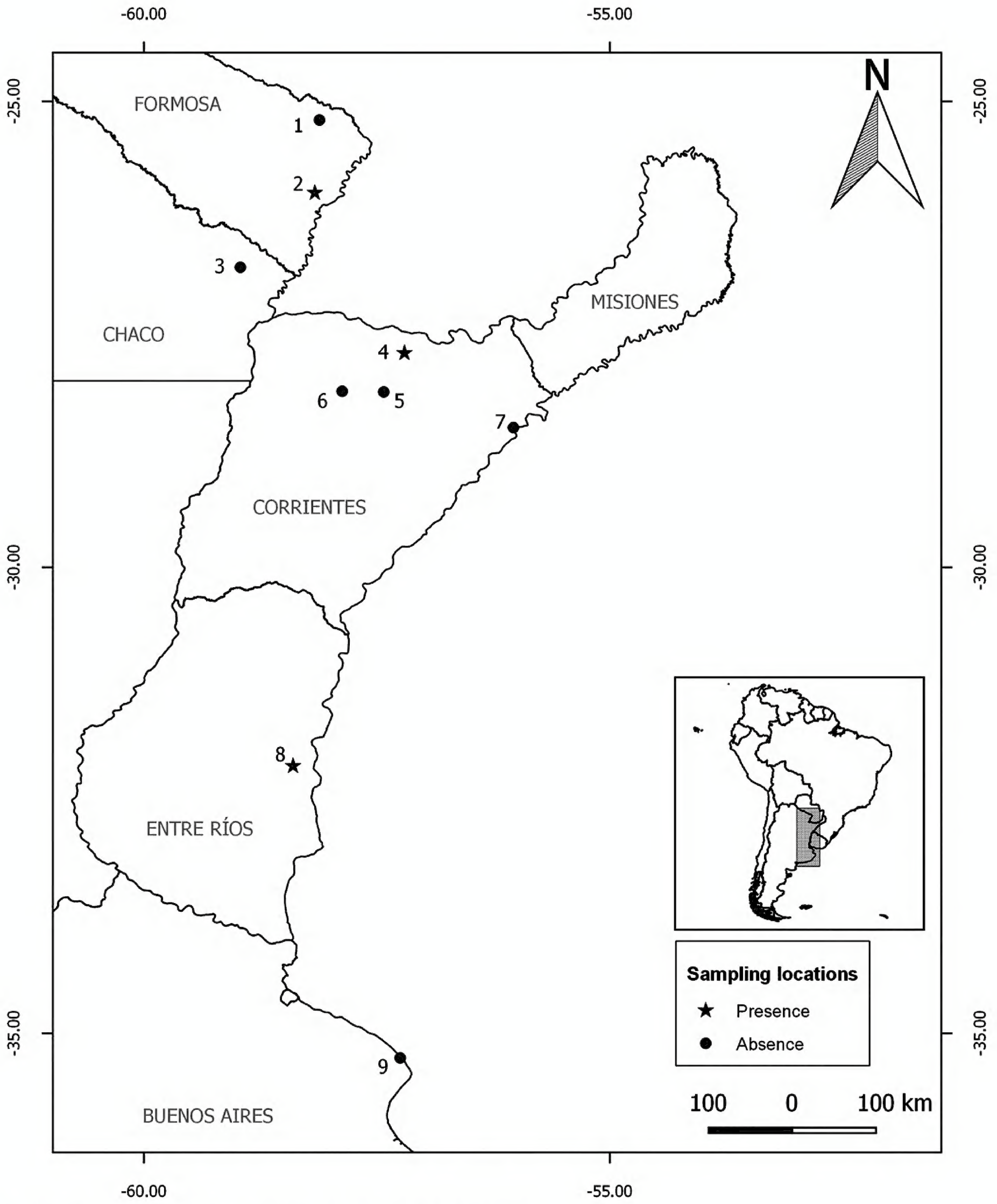

Figure 1. Absence and presence of *C. pyriformis* in each sampled localities.

parasitizing *H. vulpinus*: body somewhat pyriform, more convex dorsally than ventrally; tegument with papillae; oral sucker with deverticles; long esophagus; caeca sinuous, extended to or beyond level of ventroterminal acetabulum (Fig. 2a–c); genital sucker and hermaphroditic duct present (Fig. 2d); genital pore bifurcal or postbifurcal; cirrus pouch dorsal to genital sucker, seminal vesicle convoluted; testes branched or deeply lobed, symmetrical in mid-region of body; ovary oval and post–testicular; vitelline glands distributed from the intertesticular region to almost the posterior end of body; uterus intercaecal, dorsal to testes; excretory vesicle small, dorsal to acetabulum; excretory pore posterodorsal to acetabulum.

Discussion

Cladorchis pyriformis is distinguished from the only other species of the genus, *Cladorchis asper* (Diesing, 1838), a parasite of Tapir, *Tapirus terrestris* (Linnaeus, 1758), from Brazil by the absence of papillae on the external surface of the posterior sucker (Prudhoe 1949).

Table 2. Morphometrical characteristics of *Cladorchis pyriformis*.

Reference	Prudhoe 1949	Travassos Freitas and Kohn, 1969	Sutton and Lunaschi 1990	This paper	
Host	*D. agouti*	*T. terrestris* (= *T. americanus*)	*H. vulpinus* (as *H. brasiliensis vulpinus*)	*H. vulpinus*	*H. chacarius*
Localities	Suriname	Brazil	Buenos Aires province, Argentina; Dto. de Artigas, Uruguay	Corrientes and Entre Ríos province, Argentina	Formosa province, Argentina
Body length	4–6	5–12	4.72 (2.3–8.2)	3.88 ± 0.30 (3.50–4.20)	
Body width	2–2.8	—	3.08 (1.4–6.1)	2.18 ± 0.22 (1.90–2.40)	
Oral sucker L/W	—	0.4–0.5 / 0.2	0.66 (0.41–1.19) / 0.67 (0.45–1.17)	0.47 ± 0.21 (0.30–0.70) / 0.80 ± 0.44 (0.50–1.30)	
Acetabulum L/W	1.5–1.8 diameter	1.5–2 diameter	1.59 (0.87–2.49) / 1.06 (0.85–2.69)	1.23 ± 0.10 (1.10–1.30) / 1.40 ± 0.14 (1.20–1.50)	
Relation Vo/Ac	—	—	1: 2.08–2.62	1:2.61 / 1:1.75	
Genital sucker L/W	—	0.7–1 diameter	0.52 (0.28–0.76) / 0.73 (0.30–1.16	0.50 ± 0.08 (0.40–0.60) / 0.58 ± 0.13 (0.40–0.70)	
Testis L/W	—	—	0.26–1.19 / 0.21 (0.14–0.42)	0.70 ± 0.28 (0.50–0.90) / 0.15 ± 0.07 (0.10–0.20)	
Ovary L/W	—	—	0.08 / 0.06	0.15 ± 0.07 (0.10–0.20) / 0.09 ± 0.01 (0.08–0.10)	
Eggs	0.13–0.14 × 0.072–0.075	0.14–0.15 × 0.07–0.08	0.151 (0.146–0.157) × 0.072 (0.071–0.075)	0.12 ± 0.004 (0.12–0.13) × 0.07 ± 0.006 (0.07–0.08)	

All measurements are given in millimeters (mm), and presented as follows: mean, standard deviations, and range in parentheses. L/W= length/ width.

It is interesting to note that since 1901, these are the only two species found within this genus, which were reported in Tapiridae, Cricetidae, and Dasyproctidae (Table 2). In addition, *C. pyriformis* was reported parasitizing to Agouti, *Dasyprocta leporina* (Linnaeus, 1758), from Surinam (Prudhoe 1949), Tapirs from Brazil (Travassos et al. 1969), and marsh rats, *H. vulpinus*, from Uruguay and Argentina (Buenos Aires province) (Sutton and Lunaschi 1990).

According to Esteban et al. (2011), in the life cycle of species of the family Cladorchiidae, cercarial encystment takes place on aquatic vegetation, on the water surface, or even on snail shells. The finding of *C. pyrifformis* in both *Holochilus* species could be associated with the environmental characteristics of streams and flood areas, in addition to the herbivorous diet consisting basically of leaves and other elements of vegetable origin (Wilson et al. 2017). *Tapirus terrestris*, the other host for both *Cladorchis* species, shares a similar habitat, occurring in areas bordering the grass, such as shrubs, marshes, lakes, and streams with herbaceous banks and islands covered with grass for nocturnal feeding (Hershkovitz 1954). Until now, *C. pyrifformis* has not been found in either of these two hosts (Tapirs and marsh rats) that currently share area in the province of Formosa.

Prudhoe (1949) reported *C. pyrifformis* in *D. leporina*, finding several morphological differences between the specimens found and those of the original description, which was explained as due to the host size. *Dasyprocta leporina* has a frugivorous diet and inhabits patches of forests within the savannahs and Atlantic lowland forests. More studies on the fauna of agouti digeneans are necessary to corroborate this host–parasite association because other digenean species, such as *Stichorchis giganteus* (Diesing, 1835) show a great similarity to *C. pyrifformis* (e.g.: acetabulum ventro-subterminal, pharynx with extramural pharyngeal sac, genital sucker with genital sphincter present) (Cameron and Reesal 1951).

Rodents present the most diverse fauna of digeneans among the micromammals and show relatively low host specificity in terms of number of host species exploited. However, the host specificity in some digenean groups appears to be relatively high in terms of the taxonomic composition of the rodent host spectrum, showing a close phylogenetic relationship (Feliú et al. 2006). For example, out of the 41 species of sigmodontine rodents studied from 14 provinces (unpublished data, MRR), *C. pyrifformis* was only found in species of the genus *Holochilus*, which indicates a high host specificity. In any case, the host specificity must also be re-evaluated, enlarging the range to species of mammals present in the same habitat, since the tapir is host of the same species of *Cladorchis*. In this way, the specificity for the environment should be checked with the parasitological analysis of other species such as *Myocastor coypus* (Molina, 1782) and *Hydrochoerus hydrochaeris* Linnaeus, 1766, among others.

Holochilus vulpinus was more frequently parasitized than *H. chacarius*, although its capture was lower

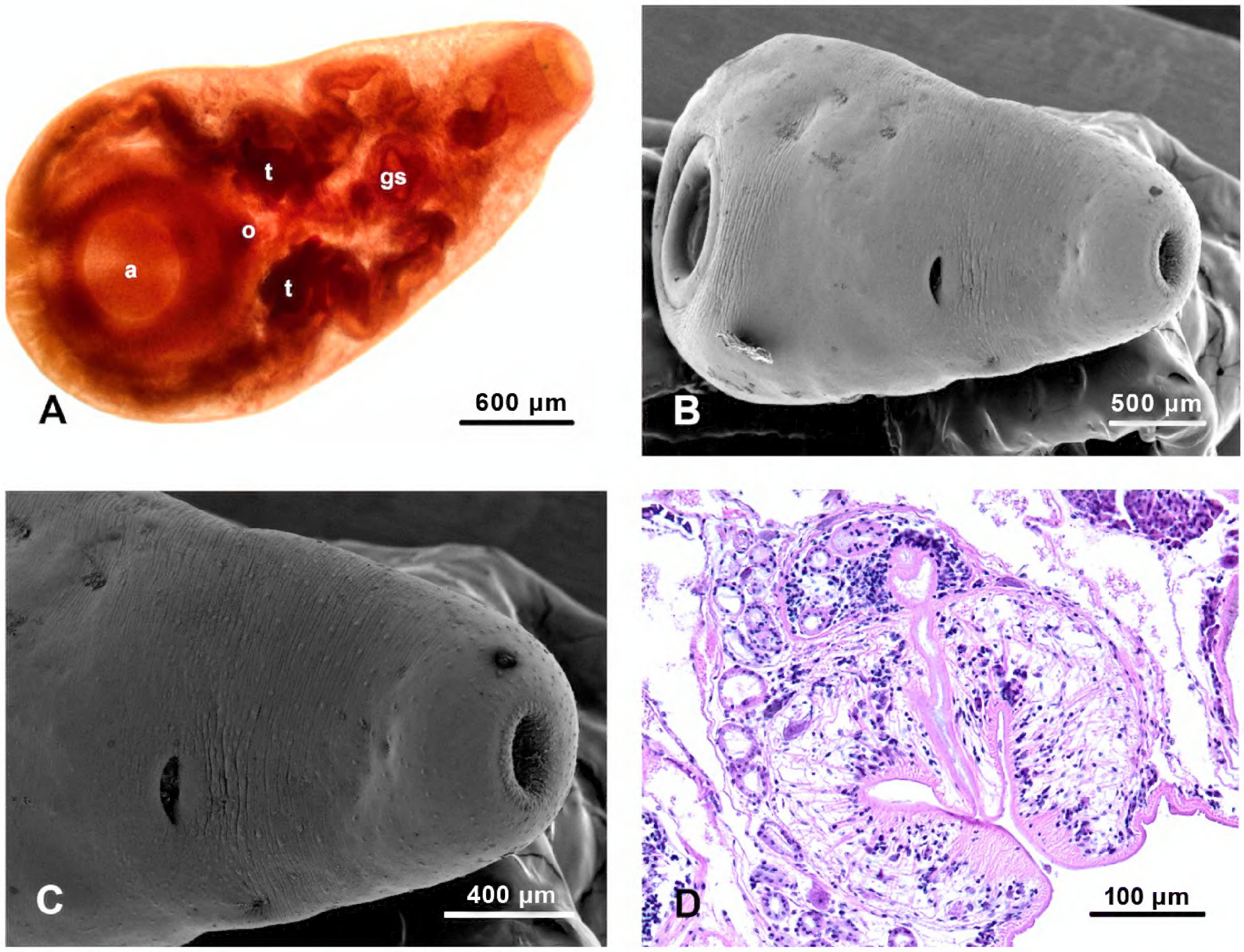

Figure 2. Micrographs obtained with polarized light and scanning electron microscope of *Cladorchis pyriformis*. **A.** Complete ventral view: testes (t), ovary (o), acetabulum (a), genital sucker (gs). **B.** Body in ventral view showing oral and genital sucker, and acetabulum. **C.** Oral and genital sucker showing detail of tegument with papillae. **D.** Detail of genital sucker with hermaphroditic duct.

compared with the latter species. Notably, the only specimen of *H. chacarius* parasitized presented the highest value of MI. In the analysis of the geographical distribution of this digenean, the highest value of P was for *H. vulpinus* in ESJP followed by VE. The values of IM and AM were similar. The majority of *H. chacarius* were collected from Laguna Blanca, Formosa province, but no infections of *C. pyriformis* were recorded from that locality. In other cases, *H. chacarius* collected did not exceed three specimens per locality (Table 1). In this context, the number of specimens captured per locality was not correlated with the number of parasitized specimens. A primary host species cannot be established with the present data, but it would be interesting to explore whether there is a preference of *C. pyriformis* for any of two species of *Holochilus*.

In this study, *C. pyriformis* is recorded for the first time in *H. chacarius* from Formosa province, and its record in *H. vulpinus* extends the geographical distribution to Entre Rios province. The continuity of the studies on Platyhelminthes, and mainly digeneans of sigmodontine rodents, will allow understanding the host and geographic distribution limits (host and environment specificity).

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Authors' Contributions

MRR collected the host specimens together with specialists in mammals and parasites (see Acknowledgements). MRR and NGM examined the viscera of specimen hosts. NGM contributed to data collection, took measurements and photographs of the specimens, and identified the species with support from all authors. NGM wrote the draft of manuscript, and all authors discussed the result and contributed to the final manuscript.

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